

Cathepsin D released by lactating rat mammary epithelial cells is involved in prolactin cleavage under physiological conditions

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Cathepsin D (CD), a lysosomal aspartyl protease, has been detected in mammary epithelial cells from lactating rat as precursor (inactive) and mature (active) forms (Markovich et al., 1985; Hernandez-Montes et al., 1999) and in milk, mainly as inactive precursor form (Larsen et al., 1996). CD was shown to cleave prolactin *in vitro* (Sinha, 1995). We asked whether in lactating mammary cells the intracellular transport and secretion of CD are regulated by prolactin and whether *in vivo* this protease is active in the catabolism of prolactin. To this aim mammary acini from lactating rats were incubated in the presence or absence of prolactin and immunogold electron microscopy co-localisation of CD and of endocytic markers was performed. In addition, the release of CD by acini, incubated in the absence or presence of brefeldin A and prolactin, and the enzymatic activity of the molecular forms of CD released in the medium were evaluated. Results show that, in the presence of prolactin, CD accumulated in endosome-like vesicles in the basal region of epithelial cells. These vesicles did not mix with endosomes internalising membrane markers. Addition of prolactin increased the release of the enzymatically active single chain form of CD, likely at the baso-lateral side of cells as shown by brefeldin A treatment of acini. These results strongly suggest that endosome-like vesicles might be basal secretory endosomes and that the release of their content might be regulated by prolactin. The enzymatically active single chain CD released by lactating rat acini, at the baso-lateral side of cells, in a conditioned medium, is able to cleave in physiological conditions the 23 kDa prolactin to generate a 16 kDa prolactin (Lkhider et al., 2004). Altogether these studies demonstrate that prolactin can itself regulate the secretion of CD, which in turn has the ability to perform outside the cell a physiological limited proteolysis of prolactin to generate in the interstitial medium a 16 kDa prolactin.